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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
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South Wales
NP10 8QQ

1.	Your reference	REP07492GB
2.	Patent application number *(The Patent Office will fill this part in)*	0401399.1 — 22 JAN 2004
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	Smart Holograms Limited 112 Hills Road Cambridge CB2 1PH
	Patents ADP number *(if you know it)*	8424273001
	If the applicant is a corporate body, give the country/state of its incorporation	United Kingdom
4.	Title of the invention	Ophthalmic Device
5.	Name of your agent *(if you have one)*	Gill Jennings & Every
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	Broadgate House 7 Eldon Street London EC2M 7LH
	Patents ADP number *(if you know it)*	745002

6. Priority: Complete this section if you are declaring priority from one or more earlier patent applications, filed in the last 12 months.

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. Divisionals, etc: Complete this section only if this application is a divisional application or resulted from an entitlement dispute (see note f)

Number of earlier UK application	Date of filing *(day / month / year)*

8. Is a Patents Form 7/77 (Statement of inventorship and of right to grant of a patent) required in support of this request? — YES

Answer YES if:

a) any applicant named in part 3 is not an inventor, or

b) there is an inventor who is not named as an applicant, or

c) any named applicant is a corporate body.

Otherwise answer NO (See note d)

9. Accompanying documents: A patent application must include a description of the invention. Not counting duplicates, please enter the number of pages of each item accompanying this form:

Continuation sheets of this form	
Description	10
Claim(s)	2
Abstract	
Drawing(s)	4 + 4

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent (Patents Form 7/77)	
Request for a preliminary examination and search (Patents Form 9/77)	
Request for a substantive examination (Patents Form 10/77)	NO
Any other documents (please specify)	

11. I/We request the grant of a patent on the basis of this application.

For the applicant
Gill Jennings & Every

Signature Date 22.01.04

12. Name, daytime telephone number and e-mail address, if any, of person to contact in the United Kingdom

PERRY, Robert Edward
020 7377 1377

Warning

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Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered YES in part 8, a Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) Part 7 should only be completed when a divisional application is being made under section 15(4), or when an application is being made under section 8(3), 12(6) or 37(4) following an entitlement dispute. By completing part 7 you are requesting that this application takes the same filing date as an earlier UK application. If you want the new application to have the same priority date(s) as the earlier UK application, you should also complete part 6 with the priority details.

Patents Form 1/77 Continuation Sheet

Our reference: REP07492GB

Applicant Details

Ciba Vision Corporation
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Duluth
Georgia
GA 30097
USA

87211930001

OPHTHALMIC DEVICE

Field of the Invention

This invention relates to an ophthalmic device comprising a holographic sensor.

Background to the Invention

Ophthalmic devices, for example contact lenses, comprising holographic elements are known. Typically, a holographic element is used to focus incoming light. The holographic element may have a programmed activating angle providing two or more optical powers. The use of a holographic element allows the user to see clear and unimpaired images, thereby overcoming many of the shortfalls of traditional simultaneous vision and translating lenses. Holographic optical inserts are described, for example, in WO-A-99/34239, WO-A-99/34244, WO-A-02/054137 and WO-A-99/34248.

The need for minimally invasive, easy-to-use glucose sensors has motivated the investigation of numerous approaches. One particular area of interest is ophthalmic glucose sensors, i.e. those for the detection of glucose in the eye. The levels of glucose in the eye are known to correlate with those in the blood, and thus blood levels of glucose can be monitored indirectly by measuring the levels in an ocular fluid such as tears.

US2003/0027240 describes an ocular insert for the detection of glucose. The insert comprises a polymerised crystalline colloidal array (PCCA) which is polymerised in a hydrogel, and a molecular recognition component capable of responding to glucose. The array has a lattice spacing that changes when the volume of the hydrogel changes, causing the diffracted wavelength of the array to change.

WO-A-9526499 discloses a holographic sensor, based on a volume hologram. This sensor comprises an analyte-sensitive matrix having an optical transducing structure disposed throughout its volume. Because of this physical arrangement of the transducer, the optical signal generated by the sensor is very sensitive to volume changes or structural rearrangements taking place in the analyte-sensitive matrix as a result of interaction or reaction with the analyte. For example, a sensor comprising a gelatin-based holographic medium may be

used to detect trypsin. Trypsin acts on the gelatin medium, irreversibly destroying the integrity of the holographic support medium. Holographic sensors may also be used to detect changes in, for example, pH.

Although sensors of the kind described in US2003/0027240 may be used to detect levels of glucose in the eye, there remains the need for ophthalmic sensors which allow for accurate, *real-time* detection of analytes such as glucose.

Summary of the Invention

The present invention is based on the realisation that holographic sensing technology, when incorporated into a contact lens or other ophthalmic device, provides an accurate yet minimally invasive method of detection of an ocular analyte. Such sensing technology may allow for the continuous, *real-time* sensing of glucose or other carbohydrates. The invention thus may improve the lives of patients having diabetes and decrease such patients' risk of developing hypoglycemia or hyperglycemia.

A first aspect of the invention is an ophthalmic device which comprises a holographic element comprising a medium and a hologram disposed throughout the volume of the medium, wherein an optical characteristic of the element changes as a result of a variation of a physical property occurring throughout the volume of the medium, wherein the variation arises as a result of interaction between the medium and an analyte present in an ocular fluid. The device may be a contact lens or an ocular implant.

Another aspect of the invention is a method of detection of an analyte in an ocular fluid, the method comprising detecting any change of the optical characteristic of the holographic element of a device of the invention with the fluid, in the eye.

Another aspect of the invention is a method for the production of a device of the invention which comprises contacting the holographic element with a contact lens, wherein the contacted regions of the element and the lens are cross-linkable; and cross-linking said regions. Preferably, at least one of the contacted regions comprises PVA, more preferably Nelfilcon.

The invention may be used for the detection of ocular analytes such as glucose or lactate. The interaction is preferably reversible so that both the interaction and reverse interaction can occur, allowing the analyte to be continuously detected, preferably in real time. The interaction is preferably a chemical reaction.

Description of the Invention

The term "glucose" as used herein refers to the known cyclic and linear forms of glucose.

The term "ophthalmic device" as used herein refers to contact lenses (both hard and soft), corneal onlays, implantable ophthalmic devices and the like.

The term "contact lens" as used herein refers to any hard or soft lens used on the eye or ocular vicinity for vision correction, diagnosis, sample collection, drug delivery, wound healing, cosmetic appearance or other ophthalmic application. The lens may be a daily-disposable, daily-wear or extended-wear lens.

The term "implantable ophthalmic device" as used herein refers to an ophthalmic device which is used in, on or about the eye or ocular vicinity. Such devices include intraocular lenses, subconjunctival lenses, intracorneal lenses, and shunts/implants (e.g. a stent or glaucoma shunt) that can rest in the cul de sac of an eye.

In a preferred embodiment, the insert is in the form of a contact lens. The lens may be manufactured using any suitable material known in the art. The lens material may be formed by the polymerisation of one or more monomers and optionally one or more prepolymers. The material may comprise a photoinitiator, visibility tinting agent, UV-blocking agent and/or a photosensitiser.

A preferred group of lens materials are prepolymers which are water-soluble and/or meltable. It is preferred that the material comprises one or more prepolymers which are in a substantially pure form (e.g. purified by ultrafiltration). Preferred prepolymers include water-soluble crosslinkable poly(vinyl alcohol) prepolymers (as described in US5583163 and US6303687); a water-soluble vinyl group-terminated polyurethane, obtainable by reacting an

isocyanate-capped polyurethane with an ethylenically unsaturated amine (primary or secondary amine) or an ethylenically unsaturated monohydroxy compound, wherein the isocyanate-capped polyurethane can be a copolymerisation product of at least one polyalkylene glycol, a compound containing at least 2 hydroxyl groups, and at least one compound with two or more isocyanate groups; derivatives of a polyvinyl alcohol, polyethyleneimine or polyvinylamine (see, for example, US5849841); a water-soluble crosslinkable polyurea prepolymer as described in US6479587; crosslinkable polyacrylamide; crosslinkable statistical copolymers of vinyl lactam, MMA and a comonomer, as disclosed in EP0655470 and US5712356; crosslinkable copolymers of vinyl lactam, vinyl acetate and vinyl alcohol, as disclosed in EP0712867 and US5665840; polyether-polyester copolymers with crosslinkable side chains, as disclosed in EP0932635; branched polyalkylene glycol-urethane prepolymers, as disclosed in EP0958315 and US6165408; polyalkylene glycol-tetra(meth)acrylate prepolymers, as disclosed in EP0961941 and US6221303; and crosslinkable polyallylamine gluconolactone prepolymers, as disclosed in WO-A-00/31150.

The lens may comprise a hydrogel material. Typically, hydrogel materials are polymeric materials which are capable of absorbing at least 10% by weight of water when fully hydrated. Hydrogel materials include poly(vinyl alcohol) (PVA), modified PVA (e.g. nelfilcon A), poly(hydroxyethyl methacrylate), poly(vinyl pyrrolidone), PVA with a poly(carboxylic acid) (e.g. carbopol), poly(ethylene glycol), polyacrylamide, polymethacrylamide, silicone-containing hydrogels, polyurethane, polyurea, and the like.

Alternatively, the device may be an implantable ophthalmic device. Glucose levels in tears may be much lower than blood glucose levels. With an implantable ophthalmic sensor, one can monitor glucose levels in aqueous humor or interstitial fluid, where glucose levels can be much higher than glucose levels in tears. Preferably, the device is in the form of a subconjunctive implant, intracorneal lens, stent or glaucoma shunt.

The holographic support medium is one in which a hologram can be made and which is capable of exhibiting one or more of the properties of the sensitive

mechanisms described herein. The hologram may be disposed on or in, part of or throughout the bulk of the volume of the support medium. Particularly in the case of a contact lens, the support medium may be an integral part of the device, e.g. the body of a lens may itself comprise a holographic support medium.

The support medium preferably comprises a native or modified matrix with viscoelastic properties which alter as a result of an interaction with an analyte species. For example, the matrix may be formed from the copolymerisation of (meth)acrylamide and/or (meth)acrylate-derived comonomers. In particular, the monomer HEMA (hydroxyethyl methacrylate) is readily polymerisable and cross-linkable. PolyHEMA is a versatile support material since it is swellable, hydrophilic and widely biocompatible.

A device in the form of a contact lens is preferably obtained by forming a holographic element and then embedding the element into a contact lens. For example, a contact lens sensor may be obtained using the following protocol:

(a) forming a polymeric holographic sensor (e.g. using phenylboronate ligands) on a glass slide or similar surface;

(b) coating a layer of polyvinylalcohol (PVA), preferably "Nelfilcon", onto the surface of the sensor, with subsequent cross-linking of the layer;

(c) extracting any toxic components from the coated sensor (e.g. using 1:1 mixture of methanol:water overnight at 40°C), followed by autoclaving;

(d) removing the sensor from the slide and cutting from it a disc of about 4mm diameter; and

(e) inserting a disc into a contact lens mould containing a contact lens and PVA, preferably Nelfilcon, then cross-linking and autoclaving the components to form the finished lens.

More than one hologram may be supported on, or in, a holographic element. Means may be provided to detect the or each variation in radiation emanating from the or each hologram, arising as a result of a variation in the or each optical characteristic. The holographic elements may be dimensioned and arranged so as to sense two independent events/species and to affect,

simultaneously, or otherwise, radiation in two different ways. Holographic elements may be provided in the form of an array.

An illuminating source of non-ionising radiation, for example visible light, may be used to observe variation(s) in the, or each, optical characteristic of the holographic element. The extent of interaction between the holographic medium and the analyte species is reflected in the degree of change of the physical property, which is detected as a variation in an optical characteristic, preferably a shift in wavelength of non-ionising radiation.

The property of the holographic element which varies may be its charge density, volume, shape, density, viscosity, strength, hardness, charge, hydrophobicity, swellability, integrity, cross-link density or any other physical property. Variation of the or each physical property, in turn, causes a variation of an optical characteristic, such as the polarisability, reflectance, refractance or absorbance of the holographic element.

The physical property that varies is preferably the size or volume of the support medium. This may be achieved by incorporating, into the support matrix, groups which undergo a reversible change upon interaction with the analyte, and cause an expansion or contraction of the support medium. The support medium may comprise a polymer or copolymer matrix, on or in which the groups are immobilised or present, e.g. in an interpenetrating network. An example of such a group is the specific binding conjugate of an analyte species. Imprinted polymers, or synthetic or biological receptors, may be used.

Another variation is in the active water, solvent or charge content of the support medium. In this case, the holographic support medium is preferably in the form of a gel.

Analyte molecules that can react with at least two functional groups in the element may form a reversible cross-link between separate parts of the support matrix, thereby altering the visco-elastic properties of the support matrix. Consequently, if present within a solvent-containing environment, and the support matrix changes, the support matrix contracts and the separation of the fringes is reduced. Specificity may be provided by ensuring that specific binding sites are provided within the medium.

One parameter determining the response of the system is the extent of cross-linking. The number of cross-linking points due to polymerisation of monomers should not be so great that complex formation between polymer and analyte-binding groups is relatively low, since the polymer film may become too rigid. This may inhibit the swelling of the support medium.

By way of example of a glucose sensor, a hydrogel-based hologram may have a support medium comprising pendant glucose groups and a lectin, preferably concanavalin A (con A). The lectin binds to the pendant glucose groups and acts as a cross-linker in the polymer structure. In the presence of freely diffusible glucose, the extent of cross-linking will decrease as glucose in solution displaces polymer-attached glucose from the binding sites on the lectin, resulting in swelling of the polymer. Volume changes in hydrogel films containing pendant glucose groups and con A can be observed using a reflection hologram. A volume change in the hydrogel alters the fringe separation of the holographic structure and can be followed as a shift in the peak wavelength of the spectral reflected response.

Water-based systems are preferred in such a holographic sensor, since they protect the lectin from exposure to organic solvents. Examples of suitable glucose components are high molecular weight dextran, and the monomers allylglucoside and 2-glucosyloxyethyl methacrylate (GEMA). Dextran, having no inherent polymerisable functionality, can be entrapped during the polymerisation of acrylamide-based monomers; allylglucoside and GEMA can be polymerised either individually or together with comonomers. The polymers are preferably prepared as thin films on glass supports.

The support medium may comprise a receptor which is capable of binding or interacting specifically with the analyte. Suitable receptors include antibodies, lectins, hormone receptors, drug receptors, enzymes, aptamers, nucleic acids, nucleic acid analogues, and fragments thereof.

A receptor may be incorporated into a support medium using any suitable method known in the art. For example, a prepolymer and receptor may comprise matching functional groups; the two components can then be covalently linked

with one another. Alternatively, a receptor may be incorporated in a vinylic monomer which a component of the lens-forming material.

A holographic glucose sensor may comprise any suitable glucose receptor, particularly one which allows a reversible change in a physical property of the support medium upon binding with glucose. For example, the support medium may comprise pendant boronate groups, such as phenylboronate or a derivative thereof. Two adjacent diol groups in glucose bind with a boronate group in a reversible condensation reaction. Thus in a holographic element, reaction of glucose with pendant boronate groups causes an expansion of the support medium, due to the formation of boronate esters. Without wishing to be bound by theory, it is believed that the boronate esters are negatively charged and effect a Donan potential, causing water to partition into the support medium. This expansion is observed as a shift in the reflectance maxima to longer wavelengths. The sensing ability of the boronate groups is strongly dependent on the molecular geometry and the aromatic species where the boronic acid group is present. Thus, glucose sensitive probes can be made with a variety of affinities, in the mM range for blood glucose, and in the μM range for tear glucose.

Similar conformational shifts occur for macrocyclic groups such as crown ethers, which reversibly bind a range of ionic species. Crown ethers are well known to reversibly bind Group I and Group II metal ions. Therefore a crown ether which is specific to an ionic analyte can be immobilised in the support medium and used to continuously monitor the presence of the analyte.

Boronate compounds, in particular phenylboronate compounds, are versatile receptors since they may be used for the detection of a variety of carbohydrates. In physiological fluids, this lack of selectivity is not a problem because most sugars are found on glycoproteins and other macromolecular structures, i.e. they are already bound and thus cannot bind to the boronate groups of the support medium. Glucose is the only sugar that is found free in relatively high concentration. Lactate (lactic acid), however, may pose a problem since it is an α-hydroxy acid which binds to boronate groups and is, in ocular fluids, generally present in a greater concentration than glucose.

The problem of lactate interference can be addressed by incorporating, in the device, a group which repels lactate. Lactate carries an overall negative charge in physiological fluids and thus, for example, the support medium may carry a group having a negative charge, the magnitude of which will be apparent to those skilled in the art. An example of such a group is 2-acrylamido-2-methyl-1-propanesulphonic acid, which can be incorporated into the support medium by copolymerisation with other monomers. Alternatively, the boronate receptor may itself carry a substantial negative charge or polarisation, e.g. by coordinating the boron atom with suitable electron-donating groups. An example of such a boronate compound is 5-fluoro-2-methylacrylamidophenylboronic acid. Another option is to attach negatively charged groups to the phenyl group of a phenylboronate receptor.

The following Examples illustrate the invention.

Example 1

A contact lens was produced according to the protocol described above. The embedded holographic element comprised 12% mol of a phenylboronate derivative (3-acrylamidophenylboronic acid).

The lens was placed into the eye of a human volunteer, who then ingested a 44g bolus of glucose. The response of the contact lens sensor was measured in terms of the shift in the wavelength of reflection. Blood glucose levels were also monitored directly using a conventional glucose sensor.

Fig. 1 shows the response of the contact lens sensor, Fig. 2 that of the blood glucose sensor. It is evident that the response of the two sensors is similar, the peak level of glucose being absorbed at around t = 25 minutes.

Example 2

An experiment similar to that of Example 1 was performed, using an ophthalmic implant comprising the sensor. The support medium was coated with Nelfilcon (Cibavision).

The experiment was conducted on a rabbit, instead of a human volunteer, the device implanted subcutaneously just below the eye. The rabbit was then anaesthetised using an xylazine-based protocol which causes blood levels of glucose to rise to a level commonly seen in diabetic patients (see Bend

Cameron *et al*, Diabetes Technology & Therapeutics). The concentration of glucose was then monitored using the implant. Again, blood levels of glucose were also monitored directly.

Fig. 3 shows the response of the holographic implant, Fig. 4 that of the blood glucose sensor. As in Example 1, the response of the two sensors is similar.

Example 3

A holographic support medium was formed by copolymerising a mixture of 12 mol% of a phenylboronate derivative, 1.5% methylene bisacrylamide (MBA) and 7 mol% 2-acrylamide-2-methyl-1-propanesulphonic acid in acrylamide. A hologram was then formed in the medium and the resulting sensor used to detect glucose in the presence of lactate in PBS at pH 7.4 and a temperature of 30°C.

Figure 5 shows the response of the sensor to varying concentrations of glucose and lactate. As can be seen, the system has become more selective for glucose over lactate. This is attributable to the sulphonic acid group repelling lactate.

Example 4

A holographic support medium was formed by copolymerising 13 mol% of a phenylboronate derivative (5-fluoro-2-methylacrylamidophenylboronic acid) and 3% MBA in acrylamide. A holographic image was then recorded in the resulting medium and the sensor used to detect glucose in the presence of lactate under the conditions specified in Example 3.

The results are shown in Figure 6. The improved selectivity to glucose over lactate is attributable to the oxygen- and nitrogen-based electron-donating groups coordinated to the boron atom of the phenylboronate receptor. These groups increase the negative change around the boron atom.

CLAIMS

1. An ophthalmic device which comprises a holographic element comprising a medium and a hologram disposed throughout the volume of the medium, wherein an optical characteristic of the element changes as a result of a variation of a physical property occurring throughout the volume of the medium, wherein the variation arises as a result of interaction between the medium and an analyte present in an ocular fluid.

2. A device according to claim 1, wherein the medium is polymeric.

3. A device according to claim 2, wherein the medium is obtainable by the polymerisation of acrylamide monomers.

4. A device according to any preceding claim, wherein the holographic element does not contain silver.

5. A device according to any preceding claim, wherein the interaction is a chemical reaction.

6. A device according to claim 5, wherein the reaction is reversible.

7. A device according to any preceding claim, which is a contact lens.

8. A device according to any of claims 1 to 6, which is implantable.

9. A device according to any preceding claim, wherein the analyte is glucose.

10. A device according to claim 9, wherein the medium comprises a boronate group.

11. A device according to claim 10, wherein the group is a phenylboronate group or derivative thereof.

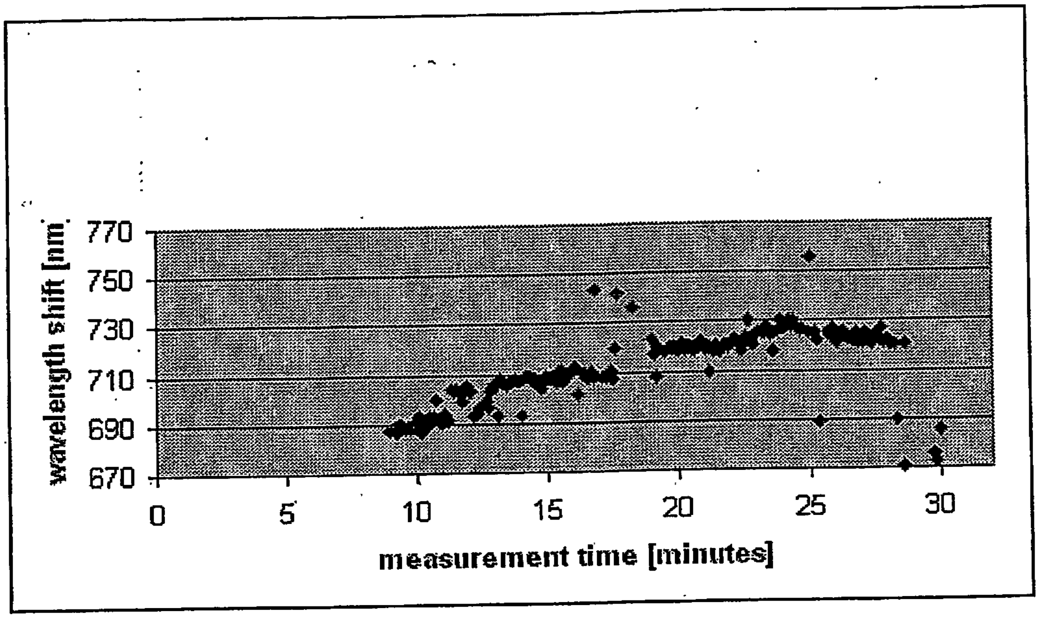
12. A device according to claim 10 or claim 11, wherein the medium comprises a group which is capable of repelling lactate, the group comprising a substantial negative change.

13. A device according to claim 12, wherein the boron atom of the boronate group carries the substantial negative change.

14. A device according to claim 12 or claim 13, wherein the medium is formed using 2-acrylamido-2-methyl-1-propane sulphonic acid and/or 5-fluoro-2-methylacrylamidophenylboronic acid.

15. A method of detection of an analyte in an ocular fluid, the method comprising detecting any change of the optical characteristic of the holographic element of a device according to any of claims 1 to 14 with the fluid, in the eye.

16. A method for the production of a device according to claim 7, which comprises contacting the holographic element with a contact lens, wherein the contacted regions of the element and the lens are cross-linkable; and cross-linking said regions.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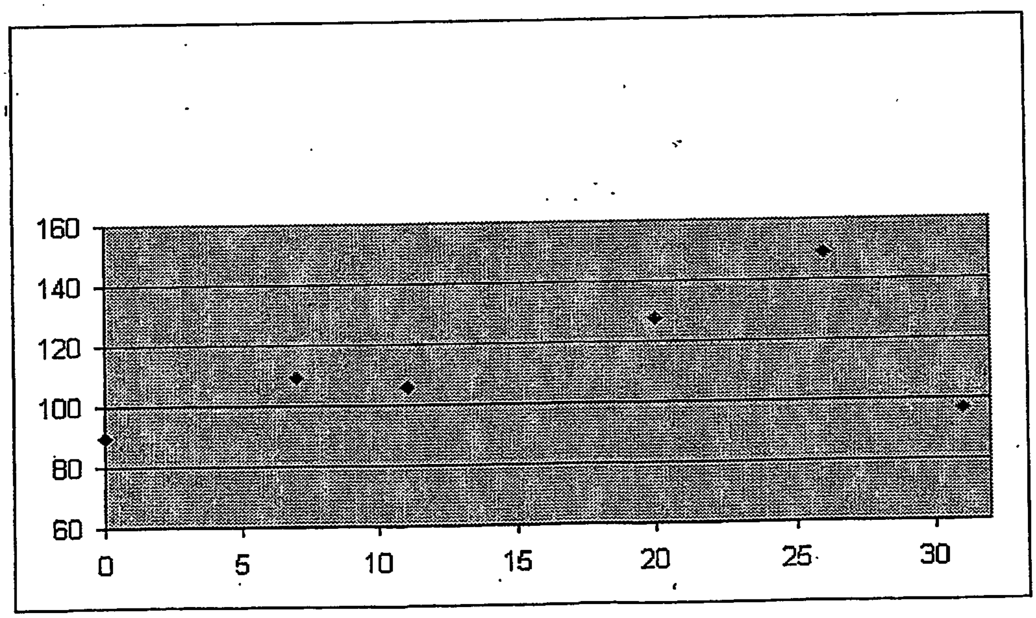
750
730
710
690
670
0
5
10
15
20
25
30
measurement time [minutes]

Fig. 1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80
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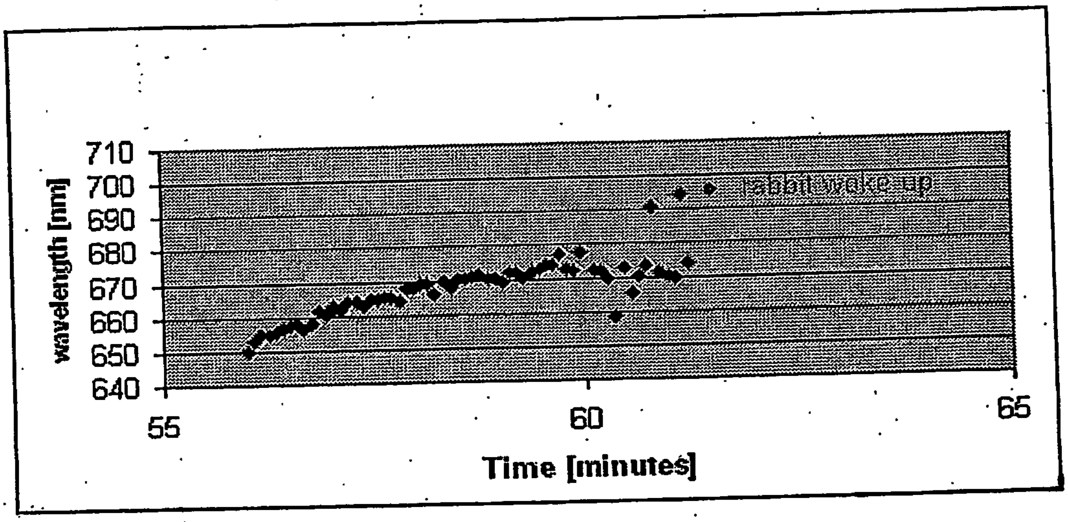
5
10
15
20
25
30

Fig. 2


710
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670
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wavelength [nm]
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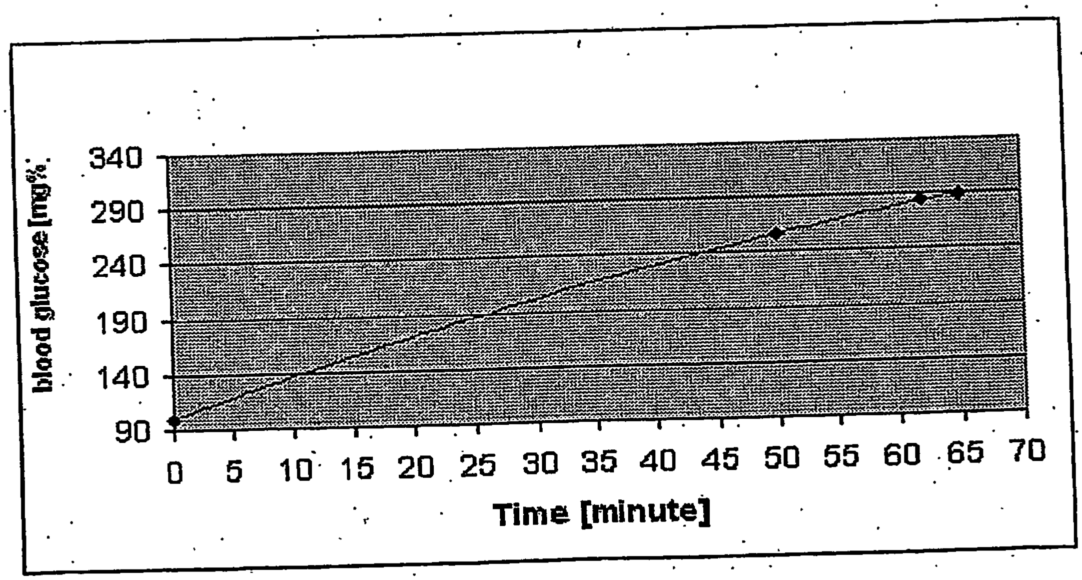
55
60
65
Time [minutes]

Fig. 3


340
290
240
190
140
90
blood glucose [mg%]
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5
10
15
20
25
30
35
40
45
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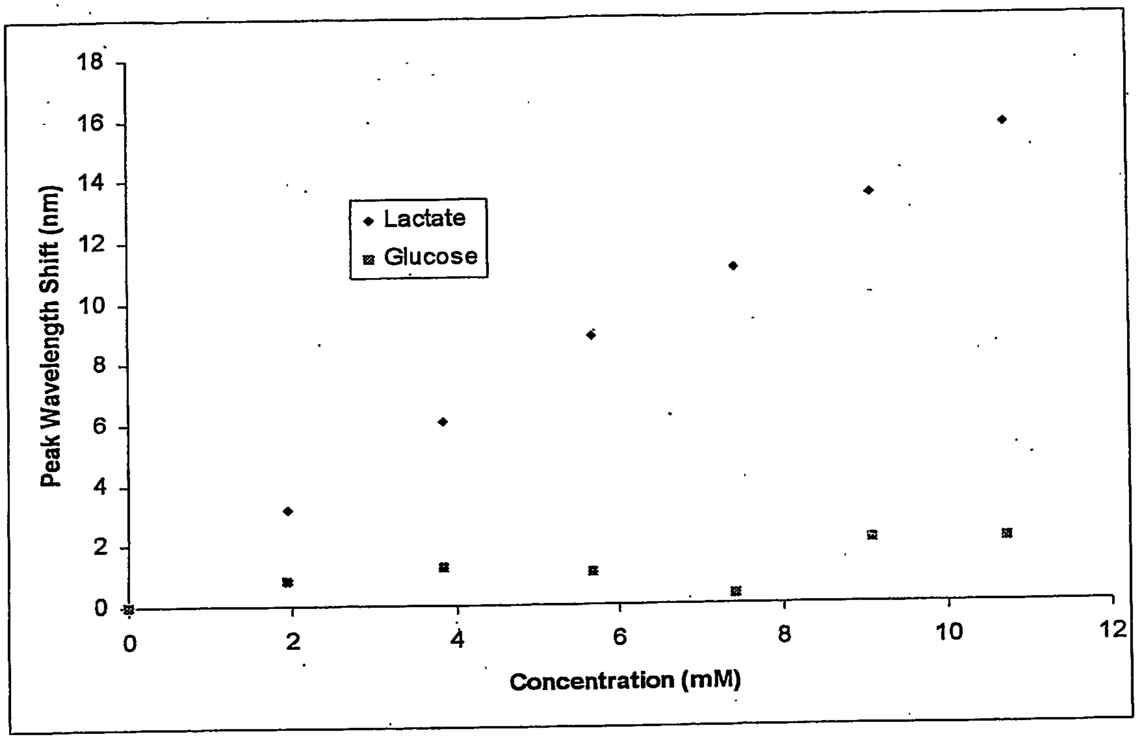
65
70
Time [minute]

Fig. 4



18
16
14
12
10
8
6
4
2
0
Peak Wavelength Shift (nm)
Lactate
Glucose
0
2
4
6
8
10
12
Concentration (mM)

Fig. 5

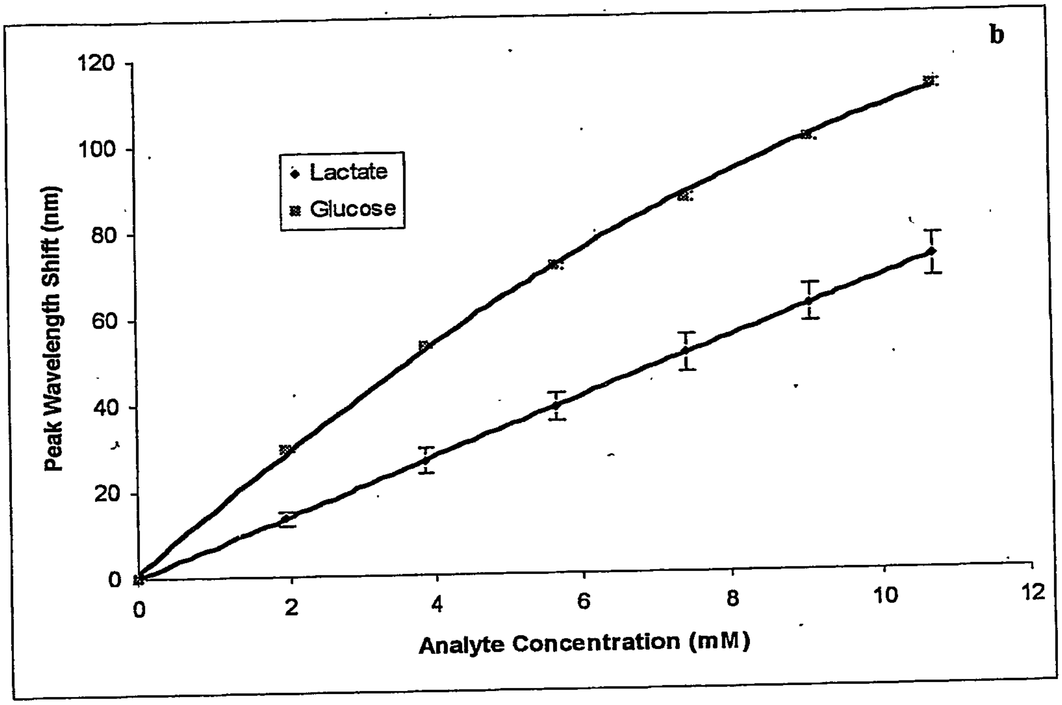

b
Peak Wavelength Shift (nm)
120
100
80
60
40
20
0
Lactate
Glucose
0
2
4
6
8
10
12
Analyte Concentration (mM)

Fig. 6

PCT/GB2004/004093